and mechanochemical properties. The model reproduces key signatures found in optical trapping studies two-kinesin complexes including observations of non-monotonic dependencies of cargo velocities and motor-microtubule unbinding rates on the applied load, and predicts that multiple kinesins have generic difficulties cooperating productively. While such behavior is influenced significantly by extrinsic factors including the spatio-temporal dependence of the applied load, the net-negative cooperative behaviors exhibited by multiple kinesins appear to be directly linked to the efficiency of kinesin's stepping mechanism, and other types of less efficient and 'weaker' processive motors are predicted to cooperate more productively. Thus, mechanochemical efficiencies of different motor types may distinguish how effectively they function as a team, and hence, how motor copy number contributes to the regulation of cargo motion. Finally, the extension of our experimental and computational approaches to studies of multiple motor dynamics inside of living cells will be discussed.

1074-Symp
Dynamics of Pairs of Processive Myosin Motors
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When two processive myosin motors (myosin V or myosin VI) are coupled together, their dynamics becomes qualitatively distinct from that of a single motor, but also from those of larger ensembles of motors. I will discuss two situations: two identical motors (myosin V, myosin V) or two motors of opposing directionality (myosin V, myosin VI). For identical motors, the dynamics of the pair is determined by an interplay between the randomness of stepping of each motor, the elasticity of the linkage and the low-force region of the force-velocity relation. For two antagonistic motors which engage in a tug of war, the outcome depends on properties of both motors close to the stall force.
Several models for processive myosins describe the dimeric motor as two identical heads, interacting solely through their mechanical connection. These models relate the properties of the dimer (e.g., step size, velocity, processivity, head-head coordination) to those of its individual heads (geometry, elasticity and kinetics). We now extend this approach to relate the properties of a pair of (identical or different) motors to those of their heads, 4 in total.

1075-Symp
Coordination of Multiple Motors Bound to Intracellular Cargos
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The movement of organelles and vesicles along the cellular cytoskeleton is often driven by multiple motor types, including kinesins, dynein, and myosins. Both in vitro and cellular studies suggest that multiple motors are bound simultaneously to intracellular cargos. For some cargos, such as late endosomes/lysosomes, we find that opposing motors may be active simultaneously, leading to stochastic directional switching best characterized as a tug-of-war. Run lengths are generally short and apparent diffusive movement predominates. For other cargos, such as autophagosomes, both kinesin and dynein motors remain stably bound but motility is highly processive in a single direction, suggesting motor activities are regulated. Intracellular transport is also regulated at the level of the track, as dictated by the complex organization of the intracellular cytoskeleton, characterized by microtubule-microtubule and microtubule-actin filament intersections as well as filament dynamics and filament-binding proteins. By analyzing motility at multiple levels, including: (1) in vitro with purified motors bound to beads at filament intersections; (2) in vitro with motors that co-purify with isolated organelles; (3) in the cell using high resolution tracking of endocytosed quantum dots; and (4) intracellular manipulation of phagocytosed beads using an optical trap, we can investigate the mechanisms that coordinate the interactions of multiple motors in intracellular organelle transport. Supported by NIH GM087253.

Minisymposium: Optical Recording of Ion Channels

1076-MiniSymp
Insights into RyRs Dysfunctions via Studies of Intracellular Calcium Signals
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Duchenne muscular dystrophy (DMD) is a striated muscle disease with severe cardiac manifestations. The *mdx* mouse, an animal model of DMD, develops dilated cardiomyopathy. Several studies associated changes in Ca^{2+} homeostasis and oxidative stress with DMD. In particular, posttranslational modifications of Ca^{2+} release channels (RyRs) increase their sensitivity, leading to augmented Ca^{2+} responses during mechanical challenges, and to cellular and mitochondrial Na^{+} overload, dysfunction and cell death. We examined whether changes in RyR function were causal for or a consequence of cardiac failure and which posttranslational modifications of RyRs drive the development of the pathology. Fluorescent indicators and imaging techniques make it possible to study the function of RyR channels in the natural cellular environment on a near-molecular level. Young *mdx* mice show no changes in cardiac performance, but do so after ~8 months. However, even myocytes from 1 month old *mdx* mice produced exaggerated Ca^{2+} sparks and waves after osmotic shock, and exhibited "hypersensitive" excitation-contraction coupling. Both were nearly abolished by antioxidants and NOX inhibitors and reduced by CaMKII but not by NOS- and PKA-inhibitors. SR Ca^{2+} load, leak or resting $[Ca^{2+}]_i$ were unchanged in young *mdx* cells. However, by the age of 4-5 months and in senescence, load was reduced, leak and resting $[Ca^{2+}]_i$ increased, indicating disease progression. By this age, all agents listed above reduced intracellular Ca^{2+} responses and prevented changes in ECC, Ca^{2+} load and leak. Thus 1) increased Ca^{2+} sensitivity of RyRs precedes and presumably contributes to the development of dystrophic cardiomyopathy and 2) oxidative stress drives its development. RyR oxidation, nitrosylation and phosphorylation, first by CaMKII followed by PKA, lead to even further sensitization. This synergistic sensitization of RyRs by several pathways results in cardiac muscle deterioration and heart failure.

1077-MiniSymp
Optical Recordings of Ca^{2+} Influx via TRPV4 and Voltage-Gated L-type Ca^{2+} Channels in Arterial Smooth Muscle
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In arterial smooth muscle, L-type Ca^{2+} channels play a critical role in multiple physiological processes including excitability, contraction, and gene expression. Recent work suggests that TRPV4 channels are also important contributors to Ca^{2+} influx in these cells. We used optical approaches to record Ca^{2+} sparklets produced by Ca^{2+} influx via TRPV4 and L-type Ca^{2+} channels in arterial myocytes under physiological conditions. We found that TRPV4 and L-type Ca^{2+} sparklet activity varies throughout the sarcolemmal of arterial myocytes. Our data suggest that these regional variations in sparklet activity arise from interactions between channels, the scaffolding protein AKAP150, and associated proteins at only a few sub-sarcolemmal regions in arterial smooth muscle cells. We will present data obtained using biochemical and optogenetic approaches to investigate the mechanisms leading to subcellular variations in TRPV4 and L-type Ca^{2+} sparklet activity as well as the functional consequences of these local Ca^{2+} signals in arterial smooth muscle. These findings will form the basis for a new model for the local control and amplification Ca^{2+} influx via TRPV4 and voltage-gated L-type Ca^{2+} channels in resistance artery smooth muscle.

1078-MiniSymp
Optical Analysis of Ryanodine Receptor Behavior *In Situ*: The Role of FKBP12.6 in Cardiomyocytes
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Since the finding of Ca^{2+} sparks (and its equivalents), optical recording has become the most important tool to study intracellular Ca^{2+}-permeable channels *in situ*, which are inaccessible otherwise by electrophysiological means. Due to the mixture of in-focus and out-of-focus events, spontaneous sparks do not quantitatively reflect the ryanodine receptor (RyR) Ca^{2+} release flux (i_{RyR}). To quantify the i_{RyR}, we activated and recorded in-focus Ca^{2+} sparks under the loose-seal patch-clamp condition, and calibrate i_{RyR} with Ca^{2+} sparklets from a single L-type Ca^{2+} channel (LCC). Using this analytical tool, we studied the role of FKBP12.6 in regulating RyR gating behavior in intact cardiomyocytes, which has been highly controversial over the last decade. We found that, in wild-type mouse ventricular myocytes, i_{RyR} exhibited a distribution with periodic quantal peaks, with each quantum of 1.05 pA representing the i_{RyR} of a single RyR. By contrast, in heart cells from FKBP12.6 knockout (FKO) mice, the quantal property of i_{RyR} was eliminated, indicating that